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Antioxidant and anti-inflammatory activities of *Frankenia triandra* (J. Rémy) extracts

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ABSTRACT

Frankenia triandra (Frankeniaceae) is a poorly studied halophyte subshrub species that grows on saline soils in the Bolivian, Argentinean and Chilean Puna, and is used by Puna inhabitants as forage and antiseptic in their folk medicine. The aim of this work is to first evaluate the antioxidant and anti-inflammatory potential (inhibitory effect on pro-inflammatory enzymes) of two hydroalcoholic extracts of *F. triandra* obtained by maceration and soxhlet extraction. Both extracts showed similar biological activity and composition (mainly polyphenolic compounds, flavonoids and phenolic acids). They exhibited an important antioxidant activity by scavenging $ABTS^{\bullet+}$ and nitrite radicals, by inhibiting β-carotene bleaching and reducing Fe^{3+}. Antioxidant properties are related to anti-inflammatory capacity, and both extracts showed a significant inhibition of hyaluronidase and two enzymes of arachidonic acid pathway (cyclooxygenase-2 and lipoxygenase). These preliminary studies are interesting since they might open the way for further studies which would allow the potential use of this plant in the treatment of chronic inflammatory diseases and as an antioxidant agent.

1. Introduction

The Puna is a highland region of the central Andes that extends through the central and Southwestern Peru, Western Bolivia, Northwestern Argentina and Northern region of Chile. The flora that grows in this region is mostly herbaceous and shrubby. A drier region can be observed in the southern area of the Argentinean and Chilean Puna, where xerophile and halophyte species are prevailing. Due to the Puna's extreme conditions (high radiation levels, extreme temperature variation during day and night, dry weather, and highly saline soils), plant species that inhabit this ecoregion have developed morphological and physiological mechanisms to survive (García and Beck, 2006). Among the physiological adaptations of plants from this eco-region, the production of secondary metabolites such as phenolic compounds, flavonoids and carotenoids can be used as defense mechanism against biotic and abiotic stress. These compounds may have remarkable benefits on human health as antioxidant, anti-inflammatory, antiseptic and antitumoral agents (Yi et al., 2005; Alberto et al., 2007; Zampini et al., 2009; Cuello et al., 2011; D'Almeida et al., 2013). This region is characterized by transitional settlements of small communities devoted to pastoralism and agropastoral activities, where plants play an important role in their daily life. Plant species are mostly used for medicinal purposes and also as forage; moreover, their use as food, firewood, tincture, among others, is also common (Villagrán et al., 2003).

Frankenia belongs to the Frankeniaceae family, which is represented by four genera of shrubby and herbaceous species that grow on highly saline soils. *Frankenia* is the most extended genera in this family and can be found in arid and semi-arid environments, growing on saline, calcareous or chalky soils (Villagrán et al., 2003; García and Beck, 2006). *Frankenia triandra* (J. Rémy), commonly known as Yareta or Yaretilla, is a halophyte subshrub species that grows on saline soils in Bolivian, Argentinian and Chilean Puna, at 3000–4500 m over sea level (m.o.s.l). This species is used by the Puna inhabitants as emergency forage, and, in folk medicine, as antiseptic (Villagrán et al., 2003). Previous studies carried out in our laboratory have demonstrated that ethanolic extract of *F. triandra* has a moderate antimicrobial activity on Gram (+) bacteria (Zampini et al., 2009). Since infection induces a localized inflammatory response in the affected tissue, we tested two extracts of *F. triandra* to determine its anti-inflammatory capacity. Moreover, studies carried out by Wided et al. (2011) on chloroformic and methanolic extracts of *Frankenia thymifolia* have shown its anti-inflammatory activity.

The main targets of anti-inflammatory drugs are inducible enzymes that produce a large number of pro-inflammatory mediators such as the enzymes from the arachidonic acid pathway (phospholipase,

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cyclooxygenase and lipoxygenase), and hyaluronidase. Non-steroidal and steroidal anti-inflammatory drugs exert their action by inhibiting these enzymes through different mechanisms (Vane and Botting, 1998). Another action mechanism of anti-inflammatory drugs is the inhibition of the reactive oxygen species generation or the scavenging of them (Werz and Steinhilber, 2005). Different studies have demonstrated a strong bond between antioxidant and anti-inflammatory properties (Rodríguez et al., 2006; Mateo Anson et al., 2011; D'Almeida et al., 2013). Nevertheless, a great amount of secondary effects have been associated to the consumption of anti-inflammatory drugs over a prolonged period of time, which increases the costs in healthcare. Hence, searching for new natural alternative sources of drugs to treat chronic inflammatory pathologies is of great interest.

Natural products play a significant role in human health in relation to the prevention and treatment of inflammatory conditions. The aim of this work is to evaluate the anti-inflammatory potential (inhibitory effect of pro-inflammatory enzymes) and antioxidant activity of two hydroalcoholic extracts of *F. triandra* obtained by different extraction methods.

2. Material and methods

2.1. Chemicals reagents

Soy lipoxygenase, 2,4-dinitrophenylhydrazine (DNPH), diphenylboric acid-ß-ethylamino ester (NP), caffeic acid, Folin–Ciocalteau reagent, aluminum chloride, quercetin dihydrate, naringenin, gallic acid, dimethylaminocinnamaldehyde (DMAC), caffeic acid, ethylenediaminetetraacetic acid (EDTA), 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ß-carotene and Griess reagent were purchased from Sigma-Aldrich (MO, USA). Hyaluronidase, linoleic acid and dimethyl sulfoxide (DMSO) were obtained from Merck (Germany). Potassium hyaluronidate was bought from Calbiochem, (USA). Triton X-100 and procyanidin B_2 were supplied by Fluka Chemical Corp. (USA). 1,2-Diheptanoilthio-glycerophosphocholine (1,2 dHGPC) and secretory phospholipase A_2 ($sPLA_2$) were obtained from bee venom and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) from Cayman Chemical Co. (MI, USA). Other chemicals were purchased from local commercial sources and they were of analytical grade quality.

2.2. Plant material

F. triandra was collected in the Laguna de Vilama (4500 m.o.s.l), in the Argentinian province of Jujuy (22°30′S 66°55′O). Botanical identification was done by Dr. *Ana* Soledad Cuello and the voucher specimen was conserved in the Fundación Miguel Lillo (487,801/LIL), its aerial parts being used in all the experiments.

2.3. Preparation of plant extracts

For ethanolic extract, 20 g of air-dried aerial parts of *F. triandra* was macerated in 100 ml of ethanol (EtOH) 80% for 7 days by shaking (40 cycles/min) at room temperature. Afterwards, extracts were filtered by using a Whatman No 1 filter paper (Sigma-Aldrich). The filtrate was dried under reduced pressure and the solid remnant was used to obtain stock solutions of 50 mg/ml in DMSO and stored at 4 °C in the dark. As for the soxhlet extract, air-dried aerial parts of *F. triandra* (65 g) were mixed with 1 l of EtOH 96%:water (1:1; *v*/v) as solvent system in a soxhlet equipment. An eight-cycle extraction was carried out, and then, the extract obtained was centrifuged for 25 min at 24,000 ×*g*. The supernatant was dried under reduced pressure to obtain a solid remnant, which was then dissolved with DMSO to obtain stock solutions of 50 mg/ml and stored at 4 °C in the dark. The extraction yields of both extracts were calculated as the ratio of the mass of the dried extract to the mass of the ground plant sample, and expressed as mg of soluble principles per g of dry weight of plant material (mg SP/g DW).

2.4. Phytochemical screening

Total phenolic compound content was determined according to Folin–Ciocalteau method (Singleton et al., 1999). Results were expressed as μg of gallic acid equivalents per mg of dry weight (μg GAE/mg DW). Non-flavonoid phenols were measured by determining the total phenol content remaining after precipitation of the flavonoids with acidic formaldehyde (Zoecklein et al., 1990). Results were expressed as μg GAE/mg DW. Flavones and flavonol content were determined according to Popova et al. (2005) method, using a 5% $AlCl_3$ solution. Flavonoid content was expressed as μg quercetin equivalents per mg of dry weight (μg QE/mg DW). Content of flavanones and dihydroflavonols was measured according to Nagy and Grançai (1996), using 1% DNPH and 70% methanol. Naringenin was used as standard and results were expressed as μg of naringenin equivalent per mg of dry weight (μg NE/mg DW). The total condensed tannin (proanthocyanidins) content was determined with DMAC according to Prior et al. (2010). Procyanidin B_2 was used as standard, and results were expressed in μg of procyanidin B_2 equivalents per mg of dry weight (μg PB_2E/mg DW).

2.5. Antioxidant activity

2.5.1. Free-radical ABTS scavenging activity

The antioxidant capacity assay was carried out according to an improved $ABTS^{\bullet+}$ method described by Re et al. (1999). One hundred microliters of an $ABTS^{\bullet+}$ solution was added to different concentrations of the extracts (25–75 μg/ml) to a final volume of 200 μl. The inhibition percentage was measured after 1 min reaction. The SC_{50} is defined as the concentration of extracts, in micrograms per milliliter (μg/ml) necessary to scavenge 50% of the $ABTS^{\bullet+}$. Quercetin was used as positive control (7–25 μg/ml).

2.5.2. β-Carotene-linoleic acid assay

Antioxidant activity of *F. triandra* extracts was determined by Wang et al. (2008) method. Different concentrations of both extracts (up to 50 μg/ml) were mixed with a β-carotene emulsion and incubated at 50 °C. The oxidation was monitored spectrophotometrically at 470 nm during 120 min. Results were compared to the control without extract. Quercetin (1.18–27.3 μg/ml) and BHT (2.3–9.1 μg/ml) were used as positive control. The concentration necessary to inhibit 50% of β-carotene bleaching (IC_{50}), expressed in micrograms per milliliter (μg/ml), was determined.

2.5.3. Nitric oxide scavenging assay

The capacity of *F. triandra* extracts to scavenge the nitric oxide released by sodium nitroprusside was determined spectrophotometically according to the method described by Govindarajan et al. (2003). Different concentrations of the extracts (100–400 μg/ml) were mixed with sodium nitroprusside (100 mM) and sodium phosphate buffer (0.2 M; pH 7.4). The reaction mixture was incubated for 60 min at 37 °C in the light. After 60 min, Griess reagent was added and incubated for 15 min in the dark. The absorbance of the formed chromophore was measured at 550 nm. SC_{50} was defined as the extract concentration necessary to scavenge 50% of nitric oxide. Ascorbic acid was used as positive control (10–100 μg/ml).

2.5.4. Iron III to iron II reductive capacity

The capacity of *F. triandra* extracts to reduce Fe (III) to Fe (II) was assessed spectrophotometrically according to Oyaizu (1986). Potassium ferricyanide 1% was mixed with different concentrations of *F. triandra* extracts, and sodium phosphate buffer (0.1 M; pH 6.3). The reaction mixture was incubated for 20 min at 50 °C, and then trichloroacetic acid 10% was added to stop the reaction. An aliquot was mixed with $FeCl_3$ 0.1%. Absorbance values at 700 nm were used to determine the concentration at which the absorbance is 0.5 for reducing power

(RC_{50}), expressed in micrograms per milliliter (μg/ml), by interpolation from a linear regression analysis (D'Almeida et al., 2013). Ascorbic (2–16 μg/ml) and gallic (0.4–3.5 μg/ml) acids were used as positive controls.

2.5.5. Metal chelating activity

Chelation of ferrous ions by plant extracts was estimated by the method of Dinis et al. (1994). Briefly, 2 mM $FeSO_4$ was added to different concentrations of the extracts (200–500 μg/ml) or to the positive control Na_2EDTA (5–20 μg/ml). The reaction was initiated by the addition of 5 mM ferrozine solution. The mixture was vigorously shaken and left to react at room temperature for 10 min. Absorbance was measured at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated.

2.6. Effect on pro-inflammatory mediators

2.6.1. Inhibition of secretory phospholipase A_2 ($sPLA_2$) activity

$sPLA_2$ activity was determined by using 1,2dHGPC and Triton X-100 as substrate (Reynolds et al., 1992). The reaction mixture contained DTNB (10 mM), enzyme $sPLA_2$ (1 mg/ml) and extracts (200 μg/ml) or commercial anti-inflammatory drugs naproxen, acetylsalicylic acid and indomethacin (50 μg/ml) in buffer Tris–HCl (10 mM; pH 8). The reaction was initiated by the addition of 1,2 dHGPC (1.66 mM) in buffer, containing $CaCl_2$ (10 mM) and KCl (100 mM), and maintained for 20 min at 25 °C. The absorbance was read at 414 nm after 20 min of reaction.

2.6.2. Inhibition of lipoxygenase (LOX) activity

LOX activity was determined spectrophotometrically according to Taraporewala and Kauffman (1990). It is based on the enzymatic oxidation of linoleic acid to the corresponding hydroperoxide. The assay was carried out with and without extracts (50 to 200 μg/ml). In order to determine the hydroperoxide produced, soy lipoxygenase-1 (948 U) was incubated with linoleic acid (50 μM) in sodium borate buffer (200 mM; pH 9.0) for 4 min at 25 °C. The anti-inflammatory effect was evaluated by calculating the inhibition percentage of hydroperoxide production from the ΔOD (optical density) values at 234 nm after 3 min of incubation. The test compound concentration producing a 50% inhibition of hydroperoxide release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis ($R^2 = 0.99$). Caffeic acid (up to 100 μg/ml) and naproxen (up to 25 μg/ml) were employed as reference drugs.

2.6.3. Inhibition of cyclooxygenase (COX) activity

The inhibitory activity of the plant extracts on COX-2 was measured by using a COX inhibitor screening assay kit (Cayman Chemical, Ann Arbor, MI), following the manufacturer's instructions, based on measuring prostaglandin (PG) by ELISA. A human recombinant COX-2 enzyme was used to form PG from arachidonic acid. The assay to obtain 100% COX activity was performed with DMSO as solvent control. The inhibitory assays were developed in the presence of 200 μg/ml plant extracts or nimesulide (0.25–2.0 μM, commercial anti-inflammatory drug). The intra- and inter-assay coefficients of variations were 5 and 10%, respectively. The effect of the different plant extracts on pro-inflammatory mediators was evaluated by calculating the inhibition percentage of PGE_2 production.

2.6.4. Inhibition of hyaluronidase activity

Hyaluronidase activity was evaluated according to the method of Lee et al. (1993) by estimating the amount of released N-acetyl glucosamine (NAGA). The reaction mixture, containing sodium acetate buffer (0.2 M; pH 4.5), hyaluronidase (57 U) and $CaCl_2$ (0.125 M), was pre-incubated for 10 min at 37 °C. Sodium hyaluronate (1.33 mg/ml) was then added both in the presence and absence of different extract concentrations (100–800 μg/ml) and incubated for 30 min at 37 °C. The activity in the absence of extract, with DMSO as solvent control, was considered to be as 100% of activity. The absorbance variation was monitored at 585 nm. Indomethacin and quercetin were employed as reference drugs (up to 200 μg/ml). The concentration inhibiting the enzymatic activity by 50% (IC_{50}) was calculated by graphic interpolation of the concentration–enzyme activity curves.

2.6.5. Stabilization of human red blood cell (HRBC) membrane

The HRBC membrane stabilization assay described by Rajakumar and Anandhan (2011) was used with slight modifications. Indomethacin and dexamethasone were used as standard drugs. Blood was collected from healthy volunteers and mixed with an equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride). Then, the blood was centrifuged and the packed cells were washed four times with isotonic NaCl solution (0.85%; pH 7.2). Afterwards, a 10% *v*/v suspension of red blood cells was made by using an isotonic NaCl solution and stored at 4 °C. Different concentrations of the extracts (400–1500 μg/ml) were mixed with phosphate buffer (0.15 M; pH 7.4), HRBC suspension and distilled water. For the 100% hemolysis control, DMSO was used as solvent control instead of the extracts. Dexamethasone and indomethacin were used as positive controls (100–400 μg/ml). All the assay mixtures were incubated at 37 °C for 30 min and then centrifuged. The hemoglobin content in the supernatant was estimated at 550 nm.

2.7. Acute toxicity test

The acute toxicity level of the extracts was studied by using *Artemia salina* as test organism. It was carried out according to Svensson et al. (2005). Ten to 12 larvae were transferred to microplates containing fresh medium and 100 to 1000 μg/ml of each extract. As control, larvae were transferred to wells without extract and DMSO as solvent control, and to wells with only artificial seawater medium. Sodium dichromate (10–40 μg/ml) was used as positive control. All the plates were incubated for 24 h at 25 °C. Afterwards, the total number of larvae and the number of immobile larvae in each well were recorded. Larvae were considered to be immobile if they did not show any forward movement for 10 s. The total number of immobile larvae was counted for each concentration and immobility was calculated.

2.8. Statistical analysis

All assays were conducted at least three times with three different sample preparations. Each experimental value is expressed as the mean ± standard deviation error (SDE). The scientific statistic software InfoStat (Student Version 2011) was used to evaluate the significance of differences between groups. Comparisons between groups were done using a Student's t-test. The criterion of statistical significance was taken as $p \leq 0.05$. For the figure, comparisons between groups were done using a one-way ANOVA with Tukey post-test at a confidence level of 95%. The criterion of statistical significance was taken as $p \leq 0.05$.

3. Results and discussion

In the present study, we employed two extraction methods to compare the extraction yield and biological activities of a continuous extraction method (soxhlet) and a static method (maceration). The extraction yields of ethanolic and soxhlet extracts were similar 167.5 and 177.6 mg SP/g DW, respectively. The advantage of the soxhlet method, as compared to maceration and other extraction procedures such as infusion, decoction and percolation, is that a large amount of drug can be extracted with a much smaller quantity of solvent, which lowers the costs and time of extraction (Tandon and Rane, 2008).

Plant species synthesize a great number of substances as product of their secondary metabolism. Studies have demonstrated that these metabolites are the main responsible for the medicinal properties of plant

species as antioxidant, anti-inflammatory, anti-carcinogenic, antibacterial, among many others (Rodríguez et al., 2006). A quantitative analysis was carried out to determine the polyphenol content (Table 1). Both extracts exhibited a similar content of total phenolic compound, flavones and flavonols, and non-flavonoid concentration. The soxhlet extract showed a higher content of flavanone-dihidroflavonol than the ethanolic extract; while the latter was richer in tannins. Studies about *Frankenia* genus are very scarce; nonetheless, a phytochemical screening conducted on methanolic and chloroformic extracts of *F. thymifolia* (from Tunisia) has revealed the presence of condensed tannins, six phenolic acids and two flavonoids (Wided et al., 2011).

Lipid peroxidation is an oxidative process that causes the disruption of essential macromolecules like DNA, proteins, phospholipids, among others. It is associated to chronic diseases, like asthma, hepatitis and cardiovascular illnesses (Barros et al., 2007). Ethanolic and soxhlet extracts showed a similar protective capacity of β-carotene oxidation, and were less active than the positive controls quercetin and BHT (Table 2). It is also remarkable that *F. triandra* extracts were more active than the chloroformic extract (SC_{50} > 1000 μg/ml) of *F. thymifolia*, but were less active than its methanolic extract (SC_{50} = 11 μg/ml) (Wided et al., 2011). The *F. trianda* extracts showed $ABTS^{\bullet+}$ radical scavenging capacity (Table 2). These results are similar to the scavenging activity obtained by Wided et al. (2011) for chloroformic and methanolic extracts of *F. thymifolia*. Both extracts were capable of reducing Fe^{3+} in a dose-dependent manner with similar potency, but they did not show a chelating capacity of Fe^{2+} up to 500 μg/ml (Table 2). It is important to mention that these extracts were more active to reduce Fe^{3+} than *F. thymifolia* extract (RC_{50} = 120 μg/ml) and chloroformic extract (RC_{50} > 1000 μg/ml) (Wided et al., 2011).

Nitric oxide may cause an oxidative damage as a result of the production of large amounts of nitrosyl ($NO^{\bullet}$), which can be converted into nitrite, or can react with superoxide radical ($O_2^{\bullet-}$) to produce peroxynitrite; both of these reactive species have a high oxidative power and oxidize proteins and lipids, causing an important damage to cells (Achike and Kwan, 2003). As shown in Table 2, both extracts have similar activity. At a concentration of 200 μg/ml, ethanolic and soxhlet extracts exhibited a moderate depurative capacity (30 and 38%, respectively), similar to the one reported for other Puna plant species that inhabit in the same environment, such as *Chiliotrichiopsis keidelii*, *Ephedra multiflora*, *Nardophyllum armatum*, *Parastrephia lepidophylla*, *P. phyliciformis* and *Tetraglochin cristatum*; whereas they were more active than *Baccharis incarum*, *Baccharis boliviensis*, *Tessaria absinthioides*, *Junellia seriphioides*, *Acantholippia deserticola* and *Chuquiraga atacamensis* (Torres Carro et al., 2015).

Different studies have demonstrated the existence of a close relationship between inflammation and the synthesis of oxidative species; furthermore, plant species that exhibit antioxidant properties tend to have anti-inflammatory properties (Radmark et al., 2007). Therefore, we analyzed the effect of *F. triandra* extracts on pro-inflammatory enzyme activity. The first enzyme of the arachidonic acid (AA) pathway is $sPLA_2$. In response to intracellular cytokines or to an increase of intracellular levels of calcium, $sPLA_2$ releases AA from the plasmatic membrane, which is then oxidized by COX or LOX to produce eicosanoids. None of the extracts was able to inhibit the activity of the pro-inflammatory enzyme $sPLA_2$. However, both extracts showed a high inhibitory capacity of LOX activity, with IC_{50} values of 134.5 ± 12.9 μg/ml for the ethanolic extract and 117.8 ± 1.8 μg/ml for the soxhlet extract. This potency was lower than that of the reference compounds such as caffeic acid and naproxen (IC_{50} of 57.0 ± 3.99 and 14.0 ± 0.70 μg/ml, respectively), but was higher than crude extracts of two *Fabiana* species, which inhabit in the same environment (Argentine Puna), (Cuello et al., 2011). A direct relationship between the inhibitory effect and the concentration was observed (Fig. 1A). This enzyme has been related to chronic diseases such as rheumatic arthritis, bronchial asthma, psoriasis; and studies have demonstrated that it is overexpressed in malignant tumor tissues (Werz and Steinhilber, 2005; Radmark et al., 2007).

Moreover, it is important to remark that the regulation of LOX activity is associated to a cyclic redox reaction of a non-heme iron located in its active site. When the enzyme is at rest, the iron is in its reduced form (Fe^{2+}), but is quickly oxidized to its ferric state (Fe^{3+}) by hydroperoxides, which allows LOX to enter into a catalytic cycle in which the iron acts as electron acceptor and donor (Radmark et al., 2007). Most of LOX inhibitors exert their action on the enzyme's active site by chelating the iron or reducing it to its ferrous form, or by scavenging electrons participating in the redox cycle of iron. Therefore, LOX inhibitors can be classified as redox active compounds, iron ligand inhibitors and non-redox inhibitors (Werz and Steinhilber, 2005). *F. triandra* extracts exerted a strong iron reducing power (Table 2) with RC_{50} values lower than their corresponding IC_{50} for LOX enzyme, which implies that both extracts, at the concentration necessary to inhibit LOX's activity in a 50%, were able to reduce 100% of free iron. Therefore, we could suggest that *F. triandra* exerts its action on LOX activity mostly by reducing the iron of its non-heme group.

Another important enzyme of the AA pathway is COX-2; it is the main enzyme in the synthesis of prostaglandins and thromboxane, important pro-inflammatory mediators that induce a local vasodilation, chemotaxis of neutrophils and increase of vascular permeability. It is an inducible isoform that is synthetized in response to cytokines, growing factors, endotoxins, lipopolysaccharides (LPS), among others, and is the main target of non-steroidal anti-inflammatory drugs (NSAIDs) (Smyth and FitzGerald, 2011). Ethanolic and soxhlet extracts exhibited a good inhibitory capacity of COX-2, reaching a 54% and 50% of inhibition at 200 μg/ml. The inhibition values are considered to be high for a crude extract (Torres Carro et al., 2015). On the other hand, the 50% inhibition was reached by the reference drug, nimesulide, at 1.25 μM (0.39 μg/ml). *F. trianda* extracts showed a similar inhibition capacity to *J. seriphioides* and *P. lepidophylla*, other Puna plant species that inhabit in the same environment than *F. triandra*; but were more active than *C. keidelii*, *E. multiflora*, *N. armatum*, *P. phyliciformis*, *T. cristatum*, *B. incarum*, *B. boliviensis*, *T. absinthioides*, *A. deserticola* and *Ch. atacamensis* (Torres Carro et al., 2015).

Hyaluronidase is a highly specific hydrolase that exerts its action by degrading hyaluronic acid, an important component of the extracellular matrix of soft connective tissue, synovial liquid, vitreous humor, among others. On inflammation, hyaluronidase increases tissue's permeability and favors the spreading of the inflammatory response through the affected tissue (El-Safory et al., 2010). Moreover, its degradation products have angiogenic properties, stimulate pro-inflammatory cytokines and immune system action, and induce the expression of inflammation-related genes (El-Safory et al., 2010). This enzyme has been found to be overexpressed in rheumatoid arthritis and osteoarthritis (El-Safory et al., 2010). In our study,

Table 1
Quantification of phenolics in ethanolic and soxhlet extracts of *Frankenia triandra*.

Sample	Total phenolics (μg GAE/mg DW)	Non flavonoid phenolics (μg GAE/mg DW)	Flavones and flavonols (μg QE/mg DW)	Flavonones and dihydroflavonols (μg NE/mg DW)	Condensed tannins (μg PB_2E/mg DW)
Ethanolic extract	172.65 ± 8.55[a]	2.27 ± 0.07[a]	17.44 ± 1.01[a]	63.00 ± 0.64[a]	11.31 ± 0.01[b]
Soxhlet extract	198.79 ± 5.56[a]	2.60 ± 0.14[a]	17.32 ± 0.56[a]	194.73 ± 5.45[b]	1.3 ± 0.01[a]

GAE: gallic acid equivalents, QE: quercetin equivalents, NE: naringenin equivalents and PB_2E: procyanidin B_2 equivalents. Values (mean ± SDE, n = 3) in the same column followed by a different letter are significantly different (Student's t-test, $p \leq 0.05$).

Table 2
Antioxidant activity of ethanolic and soxhlet extracts of *Frankenia triandra*.

Samples	ABTS$^{\bullet+}$ radical scavenging SC_{50} (μg/ml)	β-carotene/linoleic acid assay IC_{50} (μg/ml)	Nitric oxide scavenging assay SC_{50} (μg/ml)	Reducing power RC_{50} (μg/ml)
Ethanolic extract	37.22 ± 2.94[a]	41.24 ± 3.38[a]	439.20 ± 3.66[a]	15.08 ± 0.07[a]
Soxhlet extract	35.99 ± 1.31[a]	43.33 ± 2.92[a]	500.00 ± 3.89[a]	16.53 ± 0.25[a]
Quercetin	3.60 ± 0.28	7.30 ± 0.21	–	–
BHT	–	3.50 ± 0.21	–	–
Ascorbic acid	–	–	37.19 ± 0.33	5.43 ± 0.02
Gallic acid	–	–	–	1.63 ± 0.01

Nitric oxide/ABTS Free radical scavenging concentration (SC), β-carotene bleaching inhibition concentration (IC), iron reducing concentration (RC). Values (mean ± SDE, n = 3) in the same column followed by the same letter are not significantly different (Student's t-test, $p > 0.05$).

both extracts showed significant inhibition of hyaluronidase activity at all the concentrations tested in a dose-dependent manner up to 400 μg/ml (Fig. 1B). Soxhlet extract was three times more active than the ethanolic extract, with IC_{50} values of 146.3 ± 4.3 and 412.2 ± 8.9 μg/ml, respectively; and both extracts exhibited higher inhibition levels than the commercial anti-inflammatory, indomethacin (IC_{50} = 502.0 ± 7.1 μg/ml). In addition, it is worth remarking that the soxhlet extract was also more active than the positive control quercetin (IC_{50} = 340.0 ± 12.0 μg/ml). The *F. triandra* soxhlet extract exhibited a similar inhibitory potency to antiallergic drug disodium cromoglycate and catechin (IC_{50} = 140 and 180 μg/ml, respectively), as was described by Shibata et al. (2002). Furthermore, *F. triandra* extracts were more active than aqueous and ethanolic extracts of *Fabiana* species (Cuello et al., 2011).

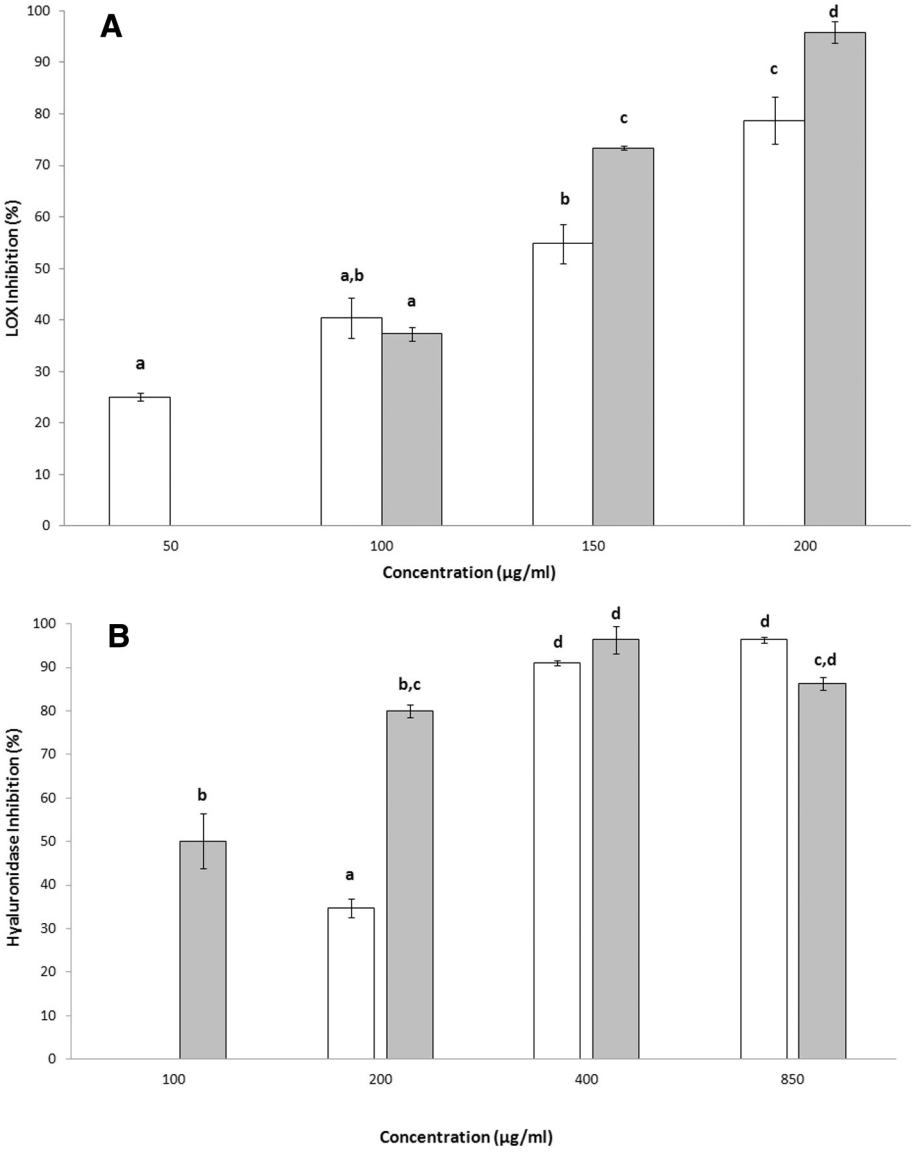

Fig. 1. Effect of different concentrations of *Frankenia triandra* ethanolic (□) and soxhlet (■) extract on lipoxigenase (**A**) and hyaluronidase (**B**) enzymes. Caffeic acid (IC_{50} 57.0 ± 2.8 μg/ml) and naproxen (IC_{50} 14.0 ± 0.5 μg/ml) as positive controls for lipoxigenase. Indomethacin (IC_{50} 502.0 ± 7.1 μg/ml) and quercetin (IC_{50} = 340.0 ± 12.0 μg/ml) as reference drug for hyaluronidase. Data (mean ± SDE, n = 3) followed by a different letter are significantly different ($p \leq 0.05$).

During the inflammatory response, neutrophils and monocytes might be damaged or suffer an apoptosis process with the consequent release of their lysosomes to the affected tissue (Crowley, 2010). These lysosomes tend to explode under the extracellular conditions, releasing their hydrolytic enzyme, which further damage the affected tissue, exacerbating and prolonging the inflammation (Crowley, 2010). Consequently, the stabilization of the lysosome membrane is a desirable property in a bid to limit the inflammatory response. Since the erythrocyte and lysosomes membrane are similar, the prevention of red blood cell lysis was taken as a measure of the extracts' anti-inflammatory activity (Mohamed Saleem et al., 2011). *F. triandra* extracts did not show a protective effect of red blood cell membrane at all the tested concentrations. Indomethacin, at concentrations of 100, 200 and 400 μg/ml, showed protection levels of 28, 38 and 32%, respectively. On the other hand, at the same concentrations, dexamethasone was less effective than indomethacin, exhibiting stabilization values of 13.9 and 4%, respectively.

Even though secondary metabolites are mostly beneficial to human health, plants can also produce a great variety of toxic substances. Therefore, preclinical toxicological studies have a great importance for the validation of the traditional use of medicinal plants. Concentrations varying from 100 to 1000 μg/ml of ethanolic and soxhlet extracts of *F. triandra* were subjected to *A. salina* toxicity test. After 24 h of incubation, none of the extracts was observed to be toxic at all the evaluated concentrations and the LD_{50} (concentration that kills 50% of the *A. salina* larvae) was not reached, while potassium dichromate exhibited a LD_{50} of 35.10 ± 0.06 μg/ml. According to Nguta et al. (2011), LD_{50} values above 1000 μg/ml are considered to be non-toxic. Accordingly, we could suggest that both *F. triandra* extracts are not toxic.

4. Conclusions

To our knowledge, this is the first report on the anti-inflammatory and antioxidant activities of *F. triandra* extracts. Both extracts showed similar chemical composition and biological activity, being the soxhlet extract more active on hyaluronidase. Even though *F. triandra* extracts were not active against $sPLA_2$, they showed a significant inhibition of the other two main enzymes of AA pathway (COX-2 and LOX). Moreover, none of the extracts was toxic. These preliminary studies are highly interesting since they might open the way for further studies which would allow the potential use of this plant in the treatment of inflammatory diseases. Other mechanisms for pharmacological activities as well as other studies to elucidate the bioactive metabolites of *F. triandra* are under investigation.

Conflict of interest

The authors have stated that there is no conflict of interest.

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